Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 11349–11353, December 1991
Immunology

Critical cytoplasmic region of the interleukin 6 signal transducer gp130 is conserved in the cytokine receptor family

(signal transduction)

MASAAKI MURAKAMI*, MASASHI NARAZAKI*, MASAHIKO HIBI*, HIDEO YAWATA*, KIYOSHI YASUKAWA†, MICHINARI HAMAGUCHI‡, TETSUYA TAGA*, AND TADAMITSU KISHIMOTO§

*Institute for Molecular and Cellular Biology, Osaka University, 1-3 Yamada-oka, Suita, Osaka 565, Japan; †Biotechnology Research Laboratory, Tosoh Corporation, Kanagawa 252, Japan; ‡Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine, Nagoya 466, Japan; and §Department of Medicine III, Osaka University Medical School, 1-1-50, Fukushima, Fukushima-ku, Osaka 553, Japan.

Contributed by Tadamitsu Kishimoto, August 27, 1991

ABSTRACT **Interleukin 6 (IL-6) signal is transduced through gp130 that associates with a complex of IL-6 and IL-6 receptor. Truncations or amino acid substitutions were introduced in the cytoplasmic region of human gp130, and the mutant cDNAs were transfected into murine interleukin 3-dependent cells to determine amino acid residues critical for generating the IL-6-mediated growth signal. In the 277-amino acid cytoplasmic region of gp130, a 61-amino acid region proximal to the transmembrane domain was sufficient for generating the growth signal. In this region, two short segments were significantly homologous with other cytokine-receptor family members. One segment is conserved in almost all members of the family, and the other is found especially in granulocyte colony-stimulating factor receptor, interleukin 2 receptor β chain, erythropoietin receptor, KH97 (a granulocyte/macrophage colony-stimulating factor receptor-associated molecule), and interleukin 3 receptor. gp130 molecules with mutations in either of these two segments could not transduce growth signal. Loss of signal-transducing ability of gp130 with such a mutation coincided with disappearance of IL-6-induced tyrosine phosphorylation of gp130.**

Interleukin 6 (IL-6) acts on a wide variety of cells and exerts multiple functions, such as growth promotion, growth inhibition, differentiation, and cell-specific gene expression (1, 2). To elucidate the mechanism of this functional pleiotropy of IL-6, we have shown that the IL-6 receptor (IL-6-R) system comprises two cell-surface molecules: an IL-6-binding protein (IL-6-R) and a signal transducer, gp130. Upon binding of IL-6, IL-6-R becomes associated extracellularly with gp130 to form high-affinity IL-6-binding sites, and gp130 transduces the signal (3, 4). Soluble IL-6-R (sIL-6-R) lacking transmembrane and cytoplasmic regions can associate with gp130 in the presence of IL-6 and mediate the IL-6 signal (3, 5).

Most receptors for cytokines involved in growth and differentiation of hematopoietic lineage cells or their associate molecules, like gp130, are structurally similar and belong to the cytokine receptor family (4, 6–8). The homologous segment in the extracellular region comprises two fibronectin type III modules and includes four conserved cysteine residues in the amino-terminal module and a Trp-Ser-Xaa-Trp-Ser motif in the other one. On this basis, it has been suggested that members of the cytokine receptor family have evolutionarily emerged from a common ancestral molecule (8, 9). For the cytoplasmic region no strict consensus sequences were seen, and no tyrosine kinase domain existed. However, the cytoplasmic regions of IL-2-R β chain, IL-3-R, IL-4-R, IL-7-R, granulocyte colony-stimulating factor receptor, erythropoietin receptor (Epo-R), and gp130 possessed a segment rich in serine residues (4, 10–16). And some sequence similarities were reported among IL-2-R β chain, Epo-R, IL-3-R (10, 12), granulocyte colony-stimulating receptor, IL-3-R, IL-4-R, and Epo-R (15). Functionally important regions in the cytoplasmic region of some cytokine-receptor family members were reported. For example, in the IL-2-R β chain, a hydrophobic amino acid in the serine-rich region was important for IL-2 signal transduction (17, 18). In the Epo-R, there was a negative regulatory domain in the carboxyl-terminal end of the cytoplasmic region, and a ≈100-amino acid segment proximal to the transmembrane domain was critical for the erythropoietin-dependent growth (19). For gp130, in addition to the serine-rich region, the consensus sequence for nucleotide binding commonly found in protein kinases and GTP-binding motif-like sequences were observed in the cytoplasmic region (4).

In our study to help understand IL-6 signal transduction through gp130, truncations or amino acid substitutions were introduced in the cytoplasmic region of gp130. First we describe the presence of a homologous region, located proximal to the transmembrane domain, which includes two segments highly conserved in the cytokine-receptor family. Using gp130 mutants, we show that, not a serine-rich region or GTP-binding motif-like sequences but instead, this homologous region is critical for generating the IL-6-mediated growth signal. This work raises the possibility that a similar mechanism might be involved in signal transduction through members of the cytokine-receptor family. We further discuss IL-6-induced tyrosine phosphorylation of gp130.

MATERIALS AND METHODS

Plasmid Construction and Transfection. pZip130 has been reported (4). For construction of plasmids pZipIC141 and pZipIC93, pUC18, including the entire human gp130 cDNA, was linearized at the cytoplasmic region with *Acc* I or *Eco*T22I, respectively, and a universal terminator (Pharmacia) was inserted. Each mutated gp130 cDNA fragment was inserted into the pZipNeoSV(X) expression vector. For construction of the other plasmids, site-directed mutagenesis was done with an oligonucleotide-directed *in vitro* mutagenesis system (Amersham), according to the manufacturer's directions. Briefly, M130, containing the entire gp130-coding sequence in M13mp18, and M130IC65, constructed from M130 to possess the termination codon at the 66th position in the cytoplasmic region of gp130, were first prepared. pZipIC10, pZipIC38, pZipIC54, pZipIC61, pZipIC65, and pZ-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: IL-2, -3, -4, -5, -6, -7, interleukins 2–7, respectively; IL-6-R, interleukin 6 receptor (attachment of an R to any interleukin abbreviation refers to its receptor); sIL-6-R, soluble IL-6-R; Epo-R, erythropoietin receptor.

ipPP were constructed from M130; pZipKK, pZipIW, pZipPOS, and pZipNEG were from M130IC65. An *Acc* II fragment containing a gp130-coding region with a specific mutation was inserted into pZipNeoSV(X). Transfectants were prepared and examined for the expression of gp130 by flow cytometric analysis (3, 4).

Internal Labeling and Immunoprecipitation. The internal labeling and immunoprecipitation were done as described (3). Briefly, [^{35}S]methionine-labeled cells (1×10^7) were stimulated with human sIL-6-R (5 μg/ml) plus IL-6 (500 ng/ml) at 37°C for 10 min. Digitonin lysates were immunoprecipitated with anti-human IL-6-R antibody MT18 (20).

Cell Proliferation Assay. Cells (1×10^4 in 0.1 ml) were cultured in triplicates with human IL-6 in the presence of sIL-6-R (2.5 μg/ml) in RPMI 1640 medium/10% fetal calf serum in 96-well microplates for 40 hr. Cells were pulse-labeled with [^{3}H]thymidine (1 nCi per well; 1 Ci = 37 GBq) for 8 hr, and the incorporated radioactivities were measured. Values obtained with sIL-6-R alone were 138 ± 21 cpm–367 ± 33 cpm, depending on transfectants used.

Immunoblot Analysis of gp130. Cells (1×10^8) were preincubated in serum-free Eagle's minimal essential medium for 4 hr and stimulated with sIL-6-R (10 μg/ml) plus IL-6 (2.5 μg/ml) for 10 min. Nonidet P-40 lysates (3) were immunoprecipitated with anti-gp130 antibody AM66 and subjected to immunoblot analysis by using polyclonal anti-phosphotyrosine antibody (21).

RESULTS

Preparation of Transfectants with gp130 Mutants. From the deduced amino acid sequence of gp130 (4), we observed the following features in the cytoplasmic region (see Fig. 1*A*): (*i*) The middle of the cytoplasmic region contains a serine-rich region, as also seen in several other cytokine receptors (IL-2-R β chain, IL-4-R, and granulocyte colony-stimulating factor receptor. (*ii*) The amino acid sequence (in single-letter code) GPGTEGQV fits the consensus sequence for nucleotide binding, GXGXXGXV, commonly found in protein kinases (27). (*iii*) Four stretches of amino acid sequences, GPGTEGQ, DAFG, NKRD, and EVSA seem partially to fit the consensus elements reported as required for GTP binding in *ras* and *ras*-related proteins [GXXXXGK, DXXG, NKXD, and EXSA (28, 29)]. (*iv*) The ≈60-amino acid segment located proximal to the transmembrane domain possessed a sequence similarity with several other members of the cytokine-receptor family (as aligned in Fig. 1*B*). In this homologous segment, two stretches of amino acids are highly conserved. One comprises a Pro-Xaa-Pro sequence and a preceding cluster of hydrophobic amino acids (box 1 in Fig. 1*B*). The Pro-Xaa-Pro sequence exists in all receptors listed in Fig. 1, except that IL-7-R possesses Pro-Xaa-His, instead. The other homologous segment (box 2) begins with a cluster of hydrophobic amino acids and ends with one or two positively charged amino acids. In the middle of box 2, some

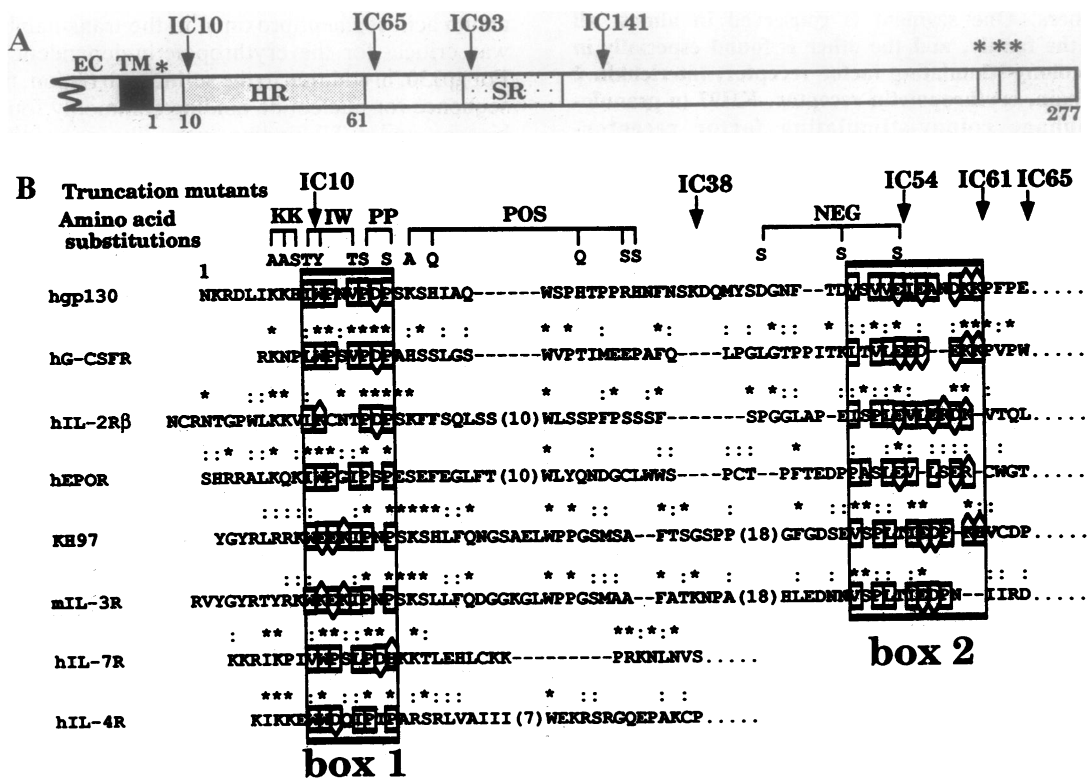

FIG. 1. Schematic structure and sequence alignment of the cytoplasmic region of human gp130. (*A*) Schematic depiction of the cytoplasmic region of human gp130. EC, extracellular region; TM, transmembrane domain; SR, serine-rich region; HR, homologous region (see Fig. 1*B*). *, GTP-binding motif-like sequences (from left, NKRD, EVSA, DAFG, and GPGTEGQV; the latter also fits the consensus for nucleotide binding). Arrows with the mutant names indicate where termination codons were introduced. (*B*) Sequence alignment of cytoplasmic region of gp130 with other cytokine-receptor family members. A part of the cytoplasmic region, located near the transmembrane domain, of the following molecules are aligned: human gp130 (4), human granulocyte colony-stimulating factor receptor (hG-CSFR) (22), human IL-2 receptor β chain (11), human erythropoietin receptor (hEPOR) (23), KH97 (24), mouse IL-3 receptor (12), human IL-7 receptor (14), and human IL-4 receptor (25). The receptors such as IL-5-R, IL-6-R, and granulocyte/macrophage colony-stimulating factor receptor having a relatively short cytoplasmic region that might not transduce the signal are omitted. Two highly conserved segments are boxed (boxes 1 and 2). In these boxes, amino acids are classified into four groups, according to the chemical characteristics of their side chains (26): nonpolar (boxed)—A, I, L, M, F, P, W, and V; polar but uncharged (unboxed)—N, C, Q, G, S, T, and Y; negatively charged ( )—D, and E; and positively charged ( )—R, H, and K. Identical amino acids with gp130 are marked as (*), and similar amino acids according to above classification are marked as (:). Gaps are introduced to maximize homology (– and numbers in parentheses). Positions of truncations and amino acid substitutions are indicated above gp130 sequence with the mutant names. The first amino acid in the cytoplasmic region is numbered 1.

negatively charged amino acids are clustered or harbored in mostly hydrophobic amino acids. The box 2 is conserved especially in gp130, granulocyte colony-stimulating factor, IL-2-R β chain, Epo-R, KH97, and IL-3-R (the last positively charged amino acid in box 2 is missing in IL-3-R). This similarity is conserved over the species (human and mouse) in each of the above receptors (data not shown). Besides the two boxes, tryptophan (W) and phenylalanine (F) residues are conserved (at positions 25 and 35, respectively, (see Fig. 1*B*).

On the basis of these structural features, we first prepared gp130 mutants carrying truncations of various numbers of amino acids from the carboxyl-terminal end. Translational termination codons were introduced at the positions indicated by arrows in Fig. 1*A*. gp130IC141 lacked three of the four GTP-binding motif-like sequences, and gp130IC93 was devoid of the serine-rich region. gp130IC65 contained a short cytoplasmic region but long enough to include the homologous region described above with four additional amino acids. gp130IC10 lacked almost all the cytoplasmic amino acids, except 10 [numbers following IC (intracytoplasmic) correspond to the number of remaining amino acids in the cytoplasmic region]. BAFB03 cells were transfected with these gp130 mutant cDNAs or the expression vector pZipneoSV(X) alone. The resulting transfectants—BAFIC141, BAFIC93, BAFIC65, BAFIC10, and BAFneoR—were obtained.

Association of Mutant gp130 Molecules with sIL-6-R and Their Function. We examined whether truncated gp130 molecules could associate with IL-6-R in the presence of IL-6. The above-mentioned five transfectants and BAF130 cells transfected with wild-type human gp130 cDNA (4) were metabolically labeled and incubated with a mixture of IL-6 and sIL-6-R because BAFB03 and its transfectants expressed no IL-6-R. Fig. 2 shows that the truncated gp130 molecules, as well as the wild-type gp130, were coprecipitated with sIL-6-R. The results indicated that gp130 mutants could normally associate with the complex of IL-6 and sIL-6-R, even when only the 10 amino acids remained in the cytoplasmic region.

We then examined whether these mutant gp130s could transduce the growth signal. Cells were incubated with sIL-6-R and IL-6, and the incorporation of the [^{3}H]thymidine was measured. Fig. 3 shows that BAFIC141, BAFIC93, BAFIC65, and BAF130 could dose-dependently respond to IL-6 in the presence of sIL-6-R, whereas BAFIC10 and BAFneoR could not. These results indicated that neither the serine-rich region nor three of the four GTP-binding motif-like sequences in gp130 were required for transduction and that the cytoplasmic homologous region of gp130 was enough for generating the IL-6-mediated growth signal in BAFB03 cells.

Function of gp130 Molecules Carrying Mutations in the Homologous Region. We further examined which part of the homologous region was critical for generating the growth signal in BAFB03 cells. Within the two conserved boxes, the Pro-Xaa-Pro sequence in box 1 is present in all receptors shown in Fig. 1*B*, except IL-7-R. The two prolines in this Pro-Xaa-Pro sequence were substituted by serines. This gp130PP mutant possessed the complete cytoplasmic region except for these two substitutions. All other amino acid substitutions were introduced in gp130IC65, which possessed 65 amino acids in the cytoplasm. In gp130IW mutant, three conserved hydrophobic amino acids, isoleucine, tryptophan, and valine, in box 1 that preceded the Pro-Xaa-Pro sequence, were substituted by threonine, tyrosine, and threonine, respectively. In gp130KK mutant, three successive positively charged amino acids, lysine, lysine, and histidine, just amino-terminal outside box 1, were substituted for by alanine, alanine, and serine, respectively. For box 2 translational termination codons were introduced at the carboxyl-terminal edge or in the middle of this box (gp130IC61 and gp130IC54, respectively). A mutant cDNA with a termination codon between boxes 1 and 2 was also prepared (gp130IC38). By using BAFB03 transfectants with the above cDNAs, these mutant gp130 molecules were revealed to retain their ability to associate with a complex of IL-6 and sIL-6-R (data not shown). From the cell-proliferation assay shown in Fig. 4, BAFPP cells with mutant gp130 cDNA, in which the highly conserved Pro-Xaa-Pro sequence was changed to Ser-Xaa-Ser but the rest of the 277-amino acid cytoplasmic region was

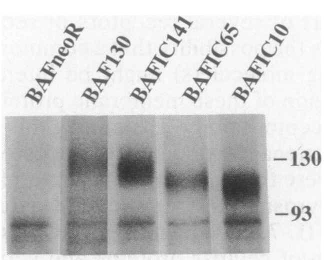

FIG. 2. Mutant molecules can associate with sIL-6-R in the presence of IL-6. BAFB03 transfectants were metabolically labeled and stimulated with sIL-6-R plus IL-6. Cells were lysed with digitonin and immunoprecipitated with the anti-IL-6-R monoclonal antibody MT18.

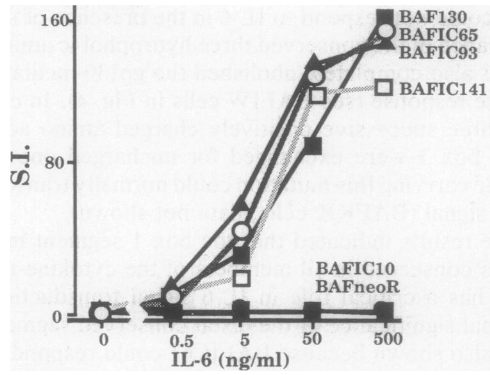

FIG. 3. Neither the serine-rich region nor three of the four GTP-binding motif-like sequences in gp130 were required for IL-6-mediated growth. BAFB03 transfectants were cultured with various concentrations of IL-6 in the presence of sIL-6-R. Incorporated [^{3}H]thymidine was measured. Data represent the stimulation index (S.I.), calculated from the incorporated radioactivity without IL-6.

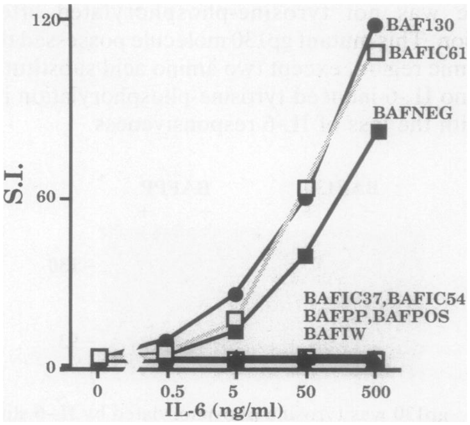

FIG. 4. Functional assay of gp130 molecules carrying mutations in the homologous region. BAFB03 transfectants expressing gp130 molecules with mutations in the homologous region were examined for the IL-6-dependent growth, as in Fig. 3. S.I., stimulation index.

intact, could not respond to IL-6 in the presence of sIL-6-R. Disturbance of the conserved three hydrophobic amino acids in box 1 also completely abolished the gp130-mediated proliferative response (see BAFIW cells in Fig. 4). In contrast, when three successive positively charged amino acids just outside box 1 were exchanged for uncharged ones, gp130 molecule carrying this mutation could normally transduce the growth signal (BAFKK cells, data not shown).

These results indicated that the box 1 segment in gp130, which is conserved in all members of the cytokine-receptor family, has a critical role in IL-6 signal transduction. The functional significance of the distal conserved segment (box 2) was also shown because BAFIC61 could respond to IL-6 in the presence of sIL-6-R, but BAFIC54 and BAFIC37 could not. These results indicated that the seven successive amino acids, IEANDKK, in box 2 were critical for generating the IL-6-mediated growth signal and that the 61-amino acid cytoplasmic portion was enough for signal transduction.

A 32-amino acid region between boxes 1 and 2 contained 9 charged amino acids. All 6 positively charged amino acids were located in the amino-terminal half of this region, and all 3 negatively charged amino acids were in the rest of the region. We prepared two mutants according to this feature. In one mutant, gp130POS, all the above-mentioned positively charged amino acids except the last lysine were substituted for by alanine, glutamine, or serine, so that the charges were lost but predicted α-helical and β-sheeted structures would not be greatly changed. In the other mutant, gp130NEG, the last 2 of the 3 negatively charged amino acids together with 1 glutamic acid residue (also a negatively charged amino acid) in box 2 were subjected to a similar substitution (see Fig. 1*B*). Fig. 4 shows that, in contrast to the fact that BAFPOS cells expressing the former mutant gp130 did not show any IL-6-dependent proliferative response, BAFNEG cells did show this response.

Phosphorylation of gp130. Some cytokines, such as IL-2, IL-3, and IL-7, phosphorylate tyrosine in cellular proteins, including the receptor (30–32). We examined whether gp130 was tyrosine-phosphorylated in response to IL-6. BAF130 cells were stimulated with a complex of IL-6 and sIL-6-R. Immunoprecipitated gp130 was immunoanalyzed by using polyclonal anti-phosphotyrosine antibody. Fig. 5 shows that gp130 molecule was tyrosine-phosphorylated after BAF130 cells were stimulated with a complex of IL-6 and sIL-6-R. When we used BAFPP cells expressing mutant gp130 in which two prolines of the highly conserved Pro-Xaa-Pro sequence were exchanged for serines, this mutant gp130 molecule was not tyrosine-phosphorylated after IL-6-stimulation. This mutant gp130 molecule possessed the entire cytoplasmic region, except two amino acid substitutions and showed no IL-6-induced tyrosine-phosphorylation in accordance with the loss of IL-6 responsiveness.

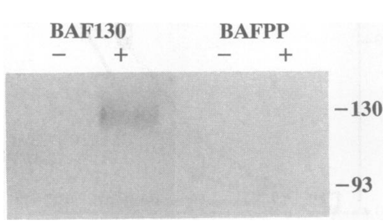

FIG. 5. gp130 was tyrosine-phosphorylated by IL-6 stimulation. BAF130 and BAFPP cells were stimulated (+) or unstimulated (−) with IL-6 plus sIL-6-R. Immunoprecipitated gp130 molecules were examined for tyrosine phosphorylation by immunoblot analysis. A comparable amount of gp130 was transferred in each lane as detected by anti-gp130 monoclonal antibody AM82 (ref. 4) (data not shown).

DISCUSSION

In this study, we showed that 61 of 277 amino acid residues in the cytoplasmic region of gp130 were sufficient to generate a growth signal in BAFB03-derived transfectants. This 61-amino acid segment did not include (*i*) the serine-rich region, (*ii*) the nucleotide-binding consensus GXGXXGXV, or (*iii*) three of the four GTP-binding motif-like sequences. The dispensability of the serine-rich region of gp130 is in contrast to the observation with IL-2-R β chain; this latter molecule could not transduce growth signal when its serine-rich region was deleted. However, the serine-rich regions in gp130 and IL-2-R β chain differed somewhat—i.e., the former includes 14 serines in the 30 successive amino acids (47%), but the latter does not include such a high serine frequency. In the serine-rich region of IL-2R β chain, a leucine residue, but not the serine residue itself, was critical for signal transduction (18). That the GTP-binding motif-like sequences in gp130 were not required for generating, at least, the growth signal may support the argument that the order of these four sequences and spacing between them differed from those in *ras* and *ras*-related proteins (4).

The 61-amino acid segment is significantly homologous with other members of the cytokine-receptor family. Although sequence similarities in the extracellular region of the cytokine-receptor family members are well documented (6–8), those in the cytoplasmic region have been little discussed. The cytoplasmic homologous part contains two highly conserved segments (boxes 1 and 2). These segments are not only structurally conserved but also are shown to be actually required for gp130 function: mutations in either of the segments abolished signal transduction. Interestingly, a previously identified amino acid in IL-2-R β chain important for the growth signal (the above-mentioned leucine; ref. 18) is located in box 2 (the latter L in this box; see Fig. 1*B*). Two of the nonfunctional gp130 mutant molecules, gp130PP and gp130IW, carry the amino acid substitution in box 1. Substitution of the two prolines by serines in the former mutant may have changed the tertiary structure of the cytoplasmic region of gp130 because proline is considered the helix-breaker and is thought to be important in protein structure (33). For example, the hinge region of immunoglobulins is rich in prolines, which may affect flexibility of the molecule and ability to bind complement (34). Substitution of the three conserved hydrophobic amino acids in box 1 (gp130IW) probably disturbed the hydrophobic interaction that may be required for folding of the important part of the cytoplasmic region. The carboxyl-terminal seven amino acids in box 2 are shown important for signal transduction. Further studies are necessary to know whether these amino acids, as well as the box 1 segment and the positively charged amino acids substituted in gp130POS, are cooperatively involved in folding of the important 61-amino acid segment or in association with downstream molecules.

The finding of two highly conserved segments in the homologous part of several receptors or receptor associate molecules raises the possibility that a common or structurally related signaling molecule(s) might be interacting with the cytoplasmic region of these membrane proteins belonging to the cytokine-receptor family. Examining this possibility may explain the functional redundancy of the cytokines. It would be of interest were the homologous part to play a role in the interaction of tyrosine kinases. Several cytokines, such as IL-2, IL-3, and IL-7, have been reported to induce tyrosine-phosphorylation of cellular proteins and activation of tyrosine kinases (30–32). In contrast, the growth factor receptors such as epidermal growth factor receptor, colony-stimulating factor 1 receptor, and platelet-derived growth factor receptor, which possess the tyrosine kinase domain in the cytoplasmic region, do not contain box 1- and box 2-like sequences. For gp130, we indicated that gp130 was tyrosine-phosphorylated upon stimulation with IL-6 plus sIL-6-R. Although direct interaction of tyrosine kinase with gp130 was not shown in BAF130 cells, the present study implies that tyrosine kinase may play a role in IL-6 signal transduction through gp130. The wild-type gp130 molecule was tyrosine-phosphorylated upon IL-6 stimulation of the cells, and loss of signal-transducing ability of gp130 coincided with disappearance of the tyrosine phosphorylation of gp130, when only two prolines in box 1 were substituted (see gp130PP in Fig. 5). A possible involvement of tyrosine kinase in the IL-6 signal-transduction pathway or a direct interaction of tyrosine kinase with gp130 has to be examined. Because the ≈60-amino acid segment of the cytoplasmic region of gp130, IL-2-R β chain, Epo-R, and IL-3-R are homologous, the signals through these molecules are suggested to be similar. In fact, these receptors all possess the ability to generate growth signal in BAFB03 cells (17, 19). However, the signaling mechanism through them differs. The parental BAFB03 cells and the IL-2-R β chain- or Epo-R-transfected cells could be grown long term in the presence of IL-3, IL-2, or erythropoietin, respectively (17–19). In contrast, IL-6 stimulation (i.e., a complex of IL-6 and sIL-6-R), even at a saturable concentration, could only support the transient growth of BAFB03 transfectants expressing gp130 (data not shown). Furthermore, transfection of murine IL-2-dependent CTLL2 cells with human gp130 cDNA did not lead the cells to the acquisition of IL-6 responsiveness (M.H., M. Saito, and M.M., unpublished data). One explanation for these observations is that a downstream signaling molecule interacting with the homologous region of each of the above receptors (including gp130) is similar but not identical.

We showed that the 61-amino acid segment of the cytoplasmic region of gp130 was sufficient for generating the IL-6-mediated growth signal. Because IL-6 is a typical pleiotropic cytokine, conceivably different parts of the cytoplasmic region of gp130 might be required for mediating the other IL-6 functions (1, 2), such as immunoglobulin production in B cells, macrophage differentiation of myeloid leukemic cells, and neural differentiation.

We thank Ms. K. Kubota and Ms. K. Ono for their excellent secretarial assistance. This study was supported by a Grant-in-Aid for Specially Promoted Research from the Ministry of Education, Science and Culture.

1. Hirano, T. & Kishimoto, T. (1990) in *Handbook of Experimental Pharmacology, Vol. 95/I Peptide Growth Factors and Their Receptors I*, ed. Sporn, M. B. & Roberts, A. B. (Springer, Berlin), pp. 633–665.
2. Kishimoto, T. (1989) *Blood* **74**, 1–10.
3. Taga, T., Hibi, M., Hirata, Y., Yamasaki, K., Yasukawa, K., Matsuda, T., Hirano, T. & Kishimoto, T. (1989) *Cell* **58**, 573–581.
4. Hibi, M., Murakami, M., Saito, M., Hirano, T., Taga, T. & Kishimoto, T. (1990) *Cell* **63**, 1149–1157.
5. Yasukawa, K., Saito, T., Fukunaga, T., Sekimori, Y., Koishihara, Y., Fukui, H., Oosugi, Y., Matsuda, T., Hirano, T., Taga, T. & Kishimoto, T. (1990) *J. Biochem.* **108**, 673–676.
6. Bazan, J. F. (1989) *Biochem. Biophys. Res. Commun.* **164**, 788–795.
7. Cosman, D., Lyman, S. D., Idzerda, R. L., Beckmann, M. P., Park, L. S., Goodwin, R. G. & March, C. J. (1990) *Trends Biol. Sci.* **15**, 265–270.
8. Bazan, J. F. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6934–6938.
9. Patthy, L. (1990) *Cell* **61**, 13–14.
10. D'Andrea, A., Fasman, G. D. & Lodish, H. F. (1989) *Cell* **58**, 1023–1024.
11. Hatakeyama, M., Tsudo, M., Minamoto, S., Kono, T., Doi, T., Miyata, T., Miyasaka, M. & Taniguchi, T. (1989) *Science* **244**, 551–556.
12. Itoh, N., Yonehara, S., Schreurs, J., Gorman, D. M., Maruyama, K., Ishii, A., Yahara, I., Arai, K. & Miyajima, A. (1990) *Science* **247**, 324–327.
13. Mosley, B., Beckmann, M. P., March, C. J., Idzerda, R. L., Gimpel, S. D., VandenBos, T., Friend, D., Alpert, A., Anderson, D., Jackson, J., Wignall, J. M., Smith, C., Gallis, B., Sims, J. E., Urdal, D., Widmer, M. B., Cosman, D. & Park, L. S. (1989) *Cell* **59**, 335–348.
14. Goodwin, R. G., Friend, D., Ziegler, S. F., Jerzy, R., Falk, B. A., Gimpel, S., Cosman, D., Dower, S. K., March, C. J., Namen, A. E. & Park, L. S. (1990) *Cell* **60**, 941–951.
15. Fukunaga, R., Ishizaka-Ikeda, E., Seto, Y. & Nagata, S. (1990) *Cell* **61**, 341–350.
16. D'Andrea, A., Lodish, H. F. & Wong, G. G. (1989) *Cell* **57**, 277–285.
17. Hatakeyama, M., Mori, H., Doi, T. & Taniguchi, T. (1989) *Cell* **59**, 837–845.
18. Mori, H., Barsoumine, E. L., Hatakeyama, M. & Taniguchi, T. (1991) *Int. Immunol.* **3**, 149–156.
19. Yoshimura, A., Longmore, G. & Lodish, H. F. (1990) *Nature (London)* **384**, 647–649.
20. Hirata, Y., Taga, T., Hibi, M., Nakano, N., Hirano, T. & Kishimoto, T. (1989) *J. Immunol.* **143**, 2900–2906.
21. Hamaguchi, M., Giandori, C. & Hanafusa, H. (1988) *Mol. Cell. Biol.* **8**, 3035–3042.
22. Fukunaga, R., Seto, Y., Mizushima, S. & Nagata, S. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 8702–8706.
23. Simon, S. J., D'Andrea, A. D., Haines, L. L. & Wong, G. G. (1990) *Blood* **76**, 31–35.
24. Hayashida, K., Kitamura, T., Gorman, D. M., Arai, K., Yokota, T. & Miyajima, A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 9655–9659.
25. Idzerda, R. L., March, C. J., Mosley, B., Lyman, S. D., Bos, T. V., Gimpel, S. D., Din, W. S., Grabstein, K. H., Widmer, M. B., Park, L. S., Cosman, D. & Beckmann, P. (1990) *J. Exp. Med.* **171**, 861–873.
26. Lehninger, A. L. (1982) *Principles of Biochemistry* (Worth, New York), pp. 95–120.
27. Hanks, S. K., Quinn, A. M. & Hunter, T. (1988) *Science* **241**, 42–52.
28. Dever, T. E., Glynias, M. J. & Merrick, W. C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1814–1818.
29. Santos, E. & Nebreda, A. R. (1989) *FASEB J.* **3**, 2151–2163.
30. Mills, G. B., May, C., McGill, M., Fung, M., Baker, M., Sutherland, R. & Greene, W. C. (1990) *J. Biol. Chem.* **265**, 3561–3567.
31. Isfort, R., Huhn, R. D., Frankelton, A. R. & Ihle, J. N. (1988) *J. Biol. Chem.* **263**, 19203–19209.
32. Uckum, F. M., Tuel-Ahlgen, L., Obuz, V., Smith, R., Dibirdik, I., Hanson, M., Langlie, M.-C. & Ledbetter, J. A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 6323–6327.
33. Chou, P. Y. & Fasman, G. D. (1978) *Adv. Enzymol.* **47**, 45–148.
34. Michaelson, T. E., Frangione, B. & Franklin, E. C. (1977) *J. Biol. Chem.* **252**, 883–889.